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PRECLINICAL STUDIES

Total Liquid Ventilation Provides Ultra-Fast Cardioprotective Cooling

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Objectives We tested whether total liquid ventilation (TLV) can be used to rapidly cool and protect the infarcting heart.

Background Decreasing myocardial temperature during ischemia is a powerful cardioprotective strategy, but clinical application has been impaired by lack of practical methodology to quickly cool the heart.

Methods We performed 30-min coronary artery occlusion/3-h reperfusion in rabbits. Upon occlusion, rabbits underwent either oxygen (Gas), normothermic liquid (Liquid Warm), or cold liquid (Liquid Cool) ventilation.

Results Left atrial chamber temperature decreased to 32.4° ± 0.2°C within 5 min of onset of cold TLV. Blood gases were within acceptable limits during TLV. In the Liquid Warm group, perfluorocarbon inhalation did not alter infarct size compared with Gas (37.7 ± 1.3% and 42.5 ± 4.9% of risk zone, respectively). However, infarction was significantly reduced in the Liquid Cool group (4.0 ± 0.5%). Cooling only during the initial 30 min of reperfusion did not reduce infarction.

Conclusions Total liquid ventilation can elicit rapid cardioprotective cooling during ischemia. (J Am Coll Cardiol 2007;49:601–5) © 2007 by the American College of Cardiology Foundation

Decreased heart temperature is a powerful cardioprotective strategy during myocardial ischemia (1–4). Even mild myocardial hypothermia that has little hemodynamic effect dramatically reduces infarct size in dogs (5), rabbits (1,2,6–9), pigs (4,10,11), and rats (12). Cooling appears to halt progression of injury during ischemia, and the sooner after occlusion it is instituted the more effective it becomes (2).

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It is uncertain whether hypothermia instituted at reperfusion protects. In one study, cooling from just before reperfusion in rabbits was not protective (1), whereas in a second study by the same researchers it was (9). Cooling pigs just before reperfusion and for 3 h thereafter was without salvage (13).

Numerous strategies for cooling the heart include skin surface cooling (14), direct epicardial cooling with bags of iced saline (7,9), infusion of cold saline in a closed circuit placed in the inferior vena cava (4), passage of the blood through a heat exchanger (2,14), and regional myocardial hypothermia with closed-circuit pericardial perfusion (8). The technique most often investigated in humans is endovascular cooling (3,15), but with a cooling rate of only 2.4°C/h the target temperature was not reached before reperfusion (15). The study was negative. An effective cardioprotective cooling intervention must cool the heart rapidly so that normothermic ischemic time before intervention is significantly reduced.

We hypothesized that total liquid ventilation (TLV) with cooled perfluorocarbon could elicit rapid cooling. These liquids have a large thermal mass and excellent gas-carrier capacity (16). The cooled perfluorocarbon should thermally equilibrate with pulmonary blood that returns directly to the left heart. Partial liquid ventilation (breathing an air-perfluorocarbon mixture) has been reported to induce hypothermia in animals (17,18), and TLV (breathing only perfluorocarbon) should, therefore, be even more effective for cooling. We evaluated TLV's ability to confer quick and cardioprotective cooling in a rabbit model of acute myocardial infarction.

Methods

Surgical preparation. This study was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington, DC, 1996) and approved by the Institutional Animal Care and Use Committee. New Zealand white rabbits anesthetized with sodium pentobarbital were prepared as previously described (2). They were ventilated through a tracheotomy with 100% O_2 and a positive pressure ventilator. After left thoracotomy, a ligature was passed around a prominent branch of

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Abbreviations and Acronyms

CAO = coronary artery occlusion

PEEP = positive end-expiratory pressure

TLV = total liquid ventilation

the left coronary artery. Temperature was measured by thermistors in the lumen of the left atrium and rectum, and arterial pressure was monitored.

Experimental protocol. Five groups of rabbits with 30-min coronary artery occlusion (CAO)/3-h reperfusion were studied. "Gas" rabbits were subjected to 100% oxygen ventilation throughout the procedure and maintained at 38°C. Groups 2 and 3 experienced TLV (tidal volume of 15 ml/kg and 6 breaths/min) throughout CAO with a target left atrial chamber temperature of either 38°C ("Liquid Warm") or 32°C ("Liquid Cool") (Fig. 1). Liquid groups returned to oxygen ventilation at reperfusion, and cooled animals spontaneously warmed. A fourth group, liquid positive end-expiratory pressure (PEEP), also had cooled TLV and was treated with 2 cm H_2O PEEP after being returned to oxygen ventilation. Finally, in the Liquid Reperfusion group cooled TLV was initiated 5 min before release of the CAO and continued for 30 min of reperfusion. After 3 h of reperfusion, hearts were removed, and after CAO the ischemic zone was negatively stained by fluoresccent microspheres and infarct identified by triphenyltetrazolium staining as previously described (2). Figure 2 illustrates the design of our liquid ventilator. We used a mixture of perfluorobutyltetrahydrofurane and perfluoropropyltetrahydropyrane (RM 101, Miteni, Milan, Italy).

Statistical analysis. Most parameters were compared by 1-way analysis of variance (ANOVA) and Student-Newman-Keuls post hoc testing. Hemodynamics were analyzed by ANOVA for repeated measures. If group differences were evident, then values at 3 critical times (i.e., baseline, after 25 min of CAO, and after 60 min of reperfusion) were compared with Tukey post hoc testing.

Results

Six animals completed the protocol in each group. Blood pressure and rate-pressure product were comparable in all groups at baseline (Table 1). Cooling decreased heart rate and rate-pressure product with gradual return to baseline after re-warming. Mean blood pressure was not significantly altered during liquid ventilation. Toward the end of the study, atelectasis occurred in the Liquid Warm and Liquid Cool groups causing hypotension in 9 of 12 animals. Atelectasis is a known complication of TLV if PEEP is not employed (16–19). In rabbits receiving 2 cm H_2O PEEP during oxygen breathing after TLV, blood pressure and oxygenation were maintained throughout reperfusion with a final mean blood pressure of 74 ± 5 mm Hg.

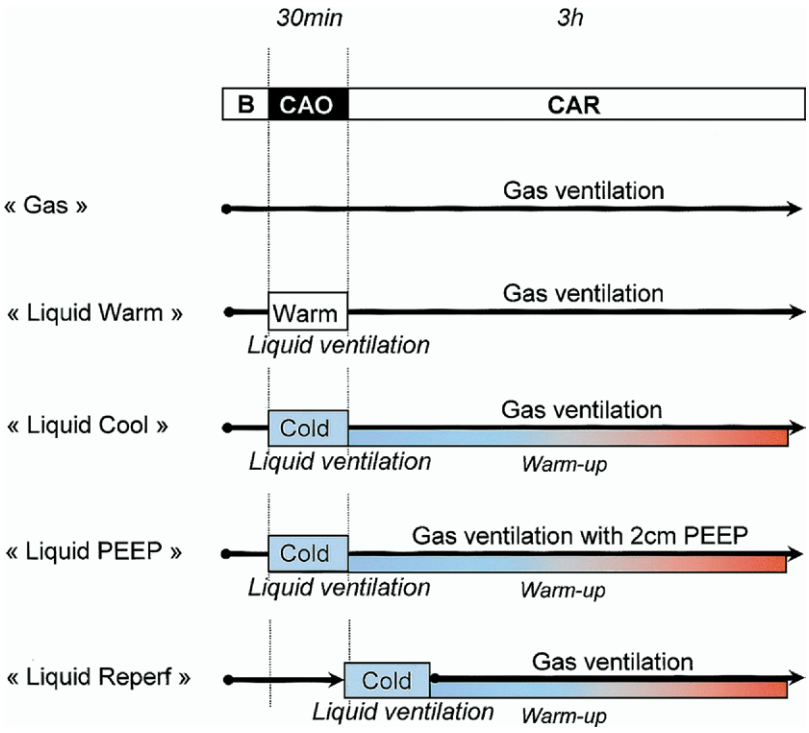

Figure 1 Experimental Protocols

B = baseline; CAO = coronary artery occlusion; CAR = coronary artery reperfusion; PEEP = positive end-expiratory pressure; Reperf = reperfusion.

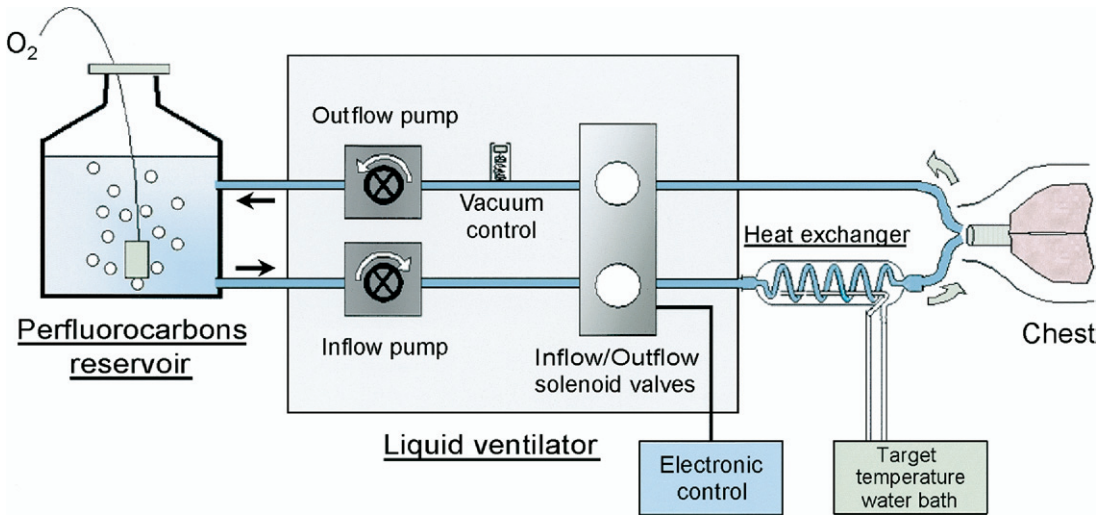

Figure 2 Diagram of the Temperature-Controlled Liquid Ventilator

Figure 3 shows the left atrial chamber and rectal temperatures for the Gas, Liquid Warm, and Liquid Cool groups. In the Liquid Cool group, TLV induced a very rapid decrease in cardiac temperature. Left atrial temperature reached target 32°C before the fifth min. Rectal temperature reflecting core temperature decreased more slowly. During reperfusion, temperature returned to baseline after 90 min. At baseline, arterial blood pH and oxygen and carbon dioxide tensions were comparable during oxygen ventilation in all groups. Liquid ventilation after 25 min of ischemia in the Liquid Warm and Liquid Cool groups was associated with normal blood pH (7.38 ± 0.02 and 7.43 ± 0.03, respectively) and oxygen (154 ± 16 and 230 ± 10 mm Hg, respectively) and carbon dioxide (37 ± 3 and 31 ± 4 mm Hg, respectively) tensions.

Infarct size. Figure 4 reveals that infarct size was dramatically reduced in the Liquid Cool and PEEP groups compared with the Liquid Warm and Gas groups ($p < 0.001$). Cooling during reperfusion had no effect on infarct size (Fig. 4).

Table 1 Hemodynamics

		Ischemia			Reperfusion		
	Baseline	5 min	15 min	25 min	5 min	30 min	60 min
Heart rate (beats/min)							
Gas	235 ± 7	247 ± 7	243 ± 10	250 ± 15	255 ± 10	251 ± 9	253 ± 8
Liquid Warm	257 ± 8	243 ± 8	223 ± 8*	238 ± 6	238 ± 12	267 ± 5	276 ± 9
Liquid Cool	245 ± 8	184 ± 5†	158 ± 6†	153 ± 4†‡§	175 ± 6†	215 ± 6*	255 ± 12
Liquid PEEP	281 ± 13‡	203 ± 10†	179 ± 6†	168 ± 7†‡	179 ± 2†	220 ± 6†	246 ± 7*
Liquid Reperfusion	262 ± 10	258 ± 8	264 ± 10	264 ± 11	198 ± 4†	168 ± 4†	224 ± 6†‡
Mean blood pressure (mm Hg)							
Gas	81 ± 4	78 ± 3	75 ± 3	73 ± 6	70 ± 6‖	74 ± 4	72 ± 5
Liquid Warm	88 ± 4	73 ± 7	61 ± 10*	62 ± 2	59 ± 5*	75 ± 4	63 ± 8‖
Liquid Cool	80 ± 4	66 ± 5	63 ± 4‖	68 ± 2‖	59 ± 5*	79 ± 4	76 ± 5
Liquid PEEP	105 ± 8‡	76 ± 10†	80 ± 6*	80 ± 7*	76 ± 5†	94 ± 7	97 ± 6‡
Liquid Reperfusion	82 ± 4	73 ± 3	74 ± 5	75 ± 5	55 ± 3†	56 ± 3†	60 ± 4†
Rate-pressure product (× 100)							
Gas	190 ± 11	193 ± 9	181 ± 8	183 ± 22	178 ± 18	186 ± 14	182 ± 16
Liquid Warm	226 ± 16	179 ± 23	139 ± 26*	162 ± 9‖	139 ± 14*	201 ± 14	175 ± 27
Liquid Cool	196 ± 14	122 ± 11†	100 ± 8†	95 ± 6†‡§	103 ± 12†	171 ± 12	196 ± 20
Liquid PEEP	297 ± 30‡	156 ± 24†	144 ± 13†	135 ± 17†	136 ± 9†	208 ± 18†	241 ± 21
Liquid Reperfusion	215 ± 17	189 ± 12	196 ± 8	200 ± 21	109 ± 8†	94 ± 6†	135 ± 11

Mean ± SEM. Statistical significance of difference between timed observation and baseline: *$p < 0.01$; †$p < 0.001$; ‖$p < 0.05$. Statistical significance of difference between Gas and Liquid groups at either baseline, 25-min ischemia, or 60-min reperfusion: ‡$p < 0.05$. Statistical significance of difference between Liquid Cool and Liquid Warm at either baseline, 25-min ischemia, or 60-min reperfusion: §$p < 0.05$.

PEEP = positive end-expiratory pressure.

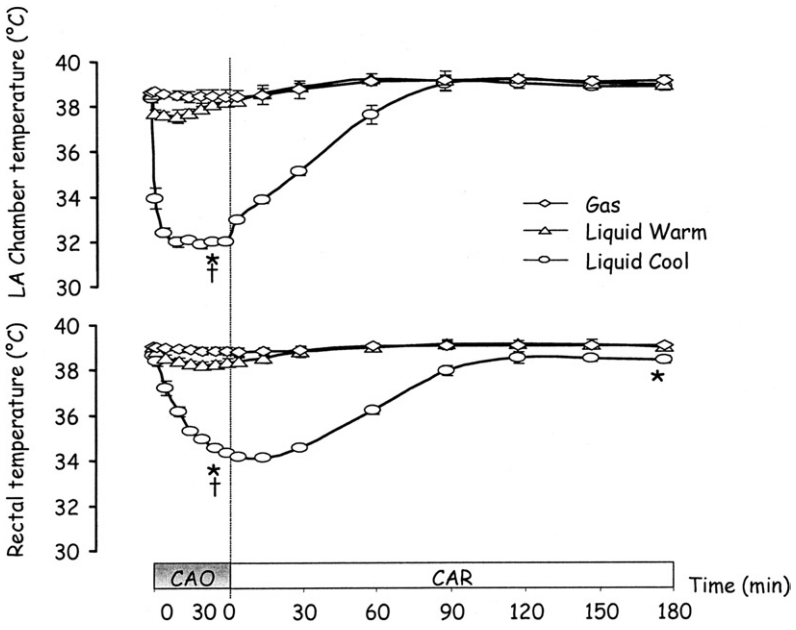

Figure 3 Cardiac and Rectal Temperatures

*p < 0.05 versus Gas; †p < 0.05 versus Liquid (comparison made at baseline, 25 min of ischemia, and 60 min of reperfusion).
LA = left atrial; other abbreviations as in Figure 1.

Discussion

Our most important finding was that TLV could elicit very rapid cardioprotective cooling in ischemic hearts. Total liquid ventilation achieved a left atrial chamber temperature of 32.4°C ± 0.2°C within 5 min. RM 101 perfluorocarbon used in these experiments has a much lower viscosity than water, but it is still higher than air. Therefore, the perfluorocarbon must be actively pumped in and out of the lungs. Studies in both animals (19,20) and humans (16) consistently report a low toxicity of perfluorocarbons during partial or TLV. These compounds are stable and inert and do not react with living tissues because the electron-rich fluorine atoms shield the underlying carbon chain (16). Although several clinical trials with partial liquid ventilation have not shown a statistically significant therapeutic benefit in acute respiratory distress syndrome (16), they did demonstrate that this material could be used safely in humans. Because of a lack of a suitable clinical indication, TLV has not yet been performed in adult humans, although it was tested in one pediatric trial (21).

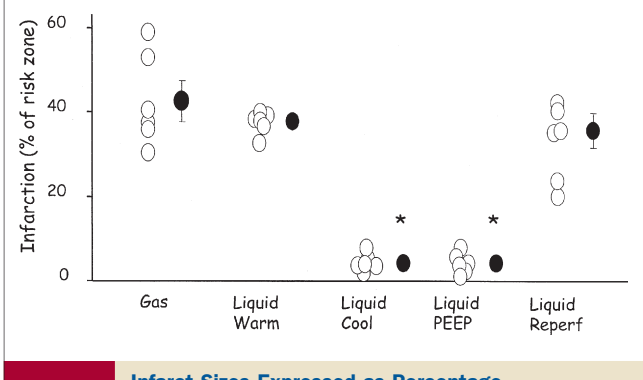

Figure 4 Infarct Sizes Expressed as Percentage of the Risk Zone Volume

Open circles = individual infarct sizes; **closed circles** = mean infarct size with SEM. *p < 0.05 versus both Gas and Liquid Warm groups. PEEP = positive end-expiratory pressure; Reperf = reperfusion.

Several reports have claimed intravenous administration of perfluorocarbon could limit infarct size (22–25), but we saw no protection with TLV unless it was accompanied by cooling. The mechanism of cardioprotection by cooling remains poorly understood, but reduced cardiac metabolism is the most likely mechanism. Hypothermia decreases the rate of high-energy phosphate (26) and glucose (27) utilization as well as lactate accumulation (27). A recent nuclear magnetic resonance study in newborn rabbit hearts confirmed that cooling favorably altered heart metabolism during ischemia/reperfusion (28). This study was designed to evaluate the effectiveness of TLV, and not to study mechanism or intracellular signaling. These issues will be addressed in the future.

Clinical implications. The accumulated data reveal that the degree of protection from cooling is essentially proportional to the reduction in normothermic ischemic time (1,2,7), whereas cooling restricted to reperfusion is futile. Temperature-controlled TLV is a promising strategy that could be instituted in patients with acute myocardial infarction being prepared for percutaneous coronary intervention.

Whether these patients would reap enough benefit to justify anesthesia and intubation depends on the delay between diagnosis and reperfusion. If the delay were more than an hour, the benefit could be substantial. In American hospitals door-to-balloon times exceeded 120 min in 41.5% of patients admitted during off-hours (29). During regular hours, 27.7% still had delays in excess of 120 min. Cooling could also be attractive in patients who have to be transferred to a different hospital for definitive intervention.

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